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Brazilian Family With Pure Autosomal Dominant Spastic Paraplegia Maps to 8q: Analysis of Muscle Beta 1 Syntrophin

P. Rocco,[1] M. Vainzof,[1,2] S.C. Froehner,[3] M.F. Peters,[4] S.K.N. Marie,[2] M.R. Passos-Bueno,[1] and M. Zatz[1]*

[1]*Centro de Estudos do Genoma Humano, Department of Biology, University of Sao Paulo, São Paulo, Brazil*
[2]*Department of Neurology, FMUSP, University of Sao Paulo, São Paulo, Brazil*
[3]*Department of Cell and Molecular Physiology, School of Medicine, University of North Carolina, Chapel Hill, North Carolina*
[4]*Department of Psychiatry, Johns Hopkins School of Medicine, Baltimore, Maryland*

The autosomal dominant hereditary spastic paraplegias (AD-HSP) are a heterogeneous group of degenerative disorders of the central motor system, characterized by progressive spasticity of the lower limbs. Five loci for pure AD-HSP have been identified to date: SPG3 at 14q, SPG4 at 2p, SPG6 at 15q, SPG8 at 8q, and more recently SPG10 at 12q. We have analyzed a Brazilian family with 16 affected individuals by pure AD-HSP who developed progressive gait disturbance with onset at age 18–26 years. Linkage analysis performed with 13 relatives (6 affected and 7 normal) excluded SPG3, SPG4, and SPG6 as candidate regions. However, positive LOD scores were obtained with markers flanking the candidate region for the SPG8 locus [maximum two point Lod score (Zmax) = 3.3 at theta = 0 for D8S1804]. In this region lies the syntrophin beta 1 gene (SNT2B1), a widely expressed dystrophin-associated protein and therefore a good positional and functional candidate for this disease. Immunohistochemical and Western Blot (WB) studies showed that the distribution, expression, and apparent molecular weight of the beta 1 syntrophin protein were comparable to those of normal control individuals. Therefore, it is unlikely that defects in this protein are related to SPG8, at least in the present family. Am. J. Med. Genet. 92:122–127, 2000.

KEY WORDS: autosomal dominant spastic paraplegia; SPG8; beta-1-syntrophin

INTRODUCTION

Autosomal dominant hereditary spastic paraplegias (AD-HSP) comprise a heterogeneous group of degenerative disorders of the central motor system, characterized by progressive spasticity of the lower limbs. The inheritance may be autosomal dominant (AD), autosomal recessive (AR), or X-linked.

Clinically, two forms of HSP can be distinguished: a pure form, with leg spasticity and weakness, and a complicated form, with other neurological or non-neurological manifestations such as optic neuropathy, retinopathy, movement disorders, dementia, epilepsy, ataxia, ichthyosis, mental retardation, and deafness. Both pure and complicated forms are heterogeneous but pure HSP is usually AD. The major neuropathological finding in this last form is axonal degeneration that involves the terminal ends of the longest fibers of the corticospinal tracts and dorsal columns [Reid, 1997].

Three recessive loci for AR-HSP have been reported and mapped to chromosomes arm 8q (SPG5) by Hentati et al. [1994], 16q (SPG7) by De Michele et al. [1998], and 15q by Martinez-Murillo et al. [1999]. The SPG7 gene product is a nuclear encoded mitochondrial metalloprotease, which was named Paraplegin. It is not known if mitochondrial abnormalities are uniquely associated with SPG7 locus or are also present in other genetic types of HSP.

X-linked spastic paraplegia was reported by us and others [Zatz et al., 1976; Keppen et al., 1987]. More

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*Correspondence to: Mayana Zatz, Centro de Estudos do Genoma Humano, Departamento de Biologia, Universidade de São Paulo, São Paulo, Brazil. E-mail: mayazatz@ib.usp.br

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recently, two distinct forms of HSP, SPG1 and SPG2, were assigned to the X-chromosome and found to be due to mutations in the L1-CAM and PLP (proteolipid protein) genes at Xq28 (Jouet et al., 1994) and Xq22 (Saugier-Veber et al., 1994), respectively. PLP is a major protein component of the central nervous system myelin and L1-CAM is a cell surface glycoprotein, which is expressed on the axons of postmitotic neurones and which is involved in neuronal migration and neurite extension.

Five loci that cause pure AD-HSP have been mapped to date: SPG3 on chromosome arm 14q [Hazan et al., 1993a,b; Gispert et al., 1995], SPG4 on chromosome arm 2p [Hazan et al., 1994; Hentati et al., 1994], SPG6 on chromosome arm 15q [Fink et al., 1995], SPG8 on chromosome arm 8q [Hedera et al., 1999a,b; Reid et al.,1999a], and more recently SPG10 on chromosome arm 12q [Reid et al., 1999b]. The protein codified by SPG4, spastin, was recently identified [Hazan et al., 1999a] but the gene product of the other AD-HSP forms is still unknown.

Among the 33 HSP kindreds reported by the Hereditary Spastic Paraplegia Working Group [Fink et al., 1996], linkage to locus SPG4 was the most common, being observed in 15 (45%) of 33 AD-HSP kindreds. Two of them (6%) were linked to SPG3 locus and one kindred (3%) was linked to the SPG6 locus. The relative proportion of SPG8 recently reported in two families of European descent is not known.

We have evaluated a Brazilian family with 16 members affected by pure spastic paraplegia with adult onset. We excluded linkage to known ADHSP loci on 2p, 14q, and 15q. The analysis with microsatellite markers flanking the candidate region for the SPG8 locus suggests linkage to this novel locus on chromosome 8q. Since the beta-1 syntrophin gene (SNT2B1) was mapped to the SPG8 locus and represents a good positional candidate for this disease, we have analyzed this protein in muscle from one affected member.

SUBJECTS AND METHODS

Subjects

This African-Brazilian family was referred to us with a diagnosis of pure spastic paraplegia. Pedigree analysis (Fig. 1) showed the existence of 16 affected members (9 males and 7 females); 12 relatives underwent a careful clinical and neurological examination. The clinical diagnosis was based on the criteria of Fink et al. [1996] at the time of blood collection and before genotyping. Deceased subjects were classified as affected or unaffected based on relatives' information.

Methods

After informed consent, DNA was extracted from blood as described elsewhere [Miller et al., 1998]. Genotype analysis was carried out by using microsatellite

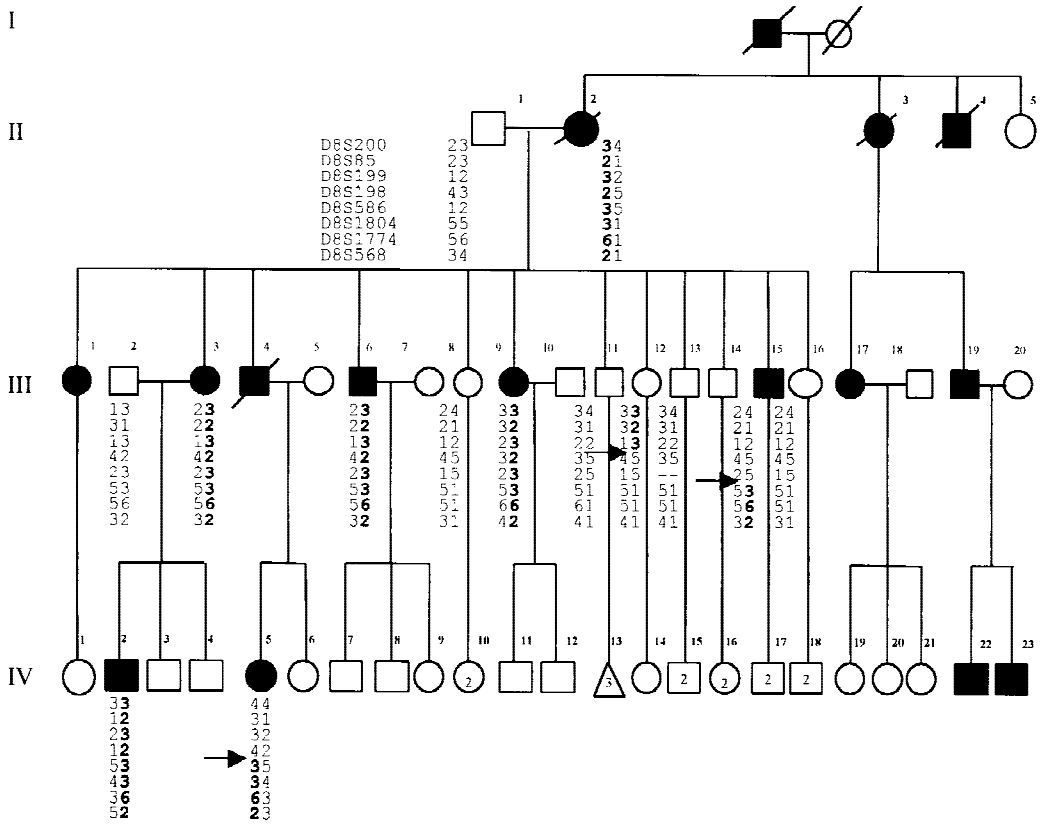

Fig. 1. Pedigree structure and haplotypes with markers from the chromosome 8q in a Brazilian AD-HSP family. The possible haplotype of individual II-2 (deceased) is boxed. The at-risk chromosome for the SPG8 locus in the family is showed in bold. The arrows show the recombinations' events placing SPG8 distal to D8S586.

polymorphic markers flanking the four known autosomal dominant loci, which were amplified by PCR, according to standard procedures. Amplifications were performed in a total volume of 15 μl in a M.J. Research termocycler under two alternative conditions: i) one primer was labeled with γ[^{32}P-ATP], by means of T4 polynucleotide kinase and ii) α[^{32}P- dCTP] was incorporated in the PCR reaction. The following program was used: 94°C for 45 sec, 55°C for 45 sec, and 72°C for 45 sec (30 cycles).

The PCR products were size-fractionated on a 6% denaturing gel electrophoresis. The gel was dried and exposed to an X-ray film at room temperature for 2–24 hr.

Linkage Analysis

Two-point linkage analysis was performed for each marker and the disease gene. The lod-scores were estimated through the MLINK subroutine of the LINKAGE program, version 5.1 [Lathrop, 1984], based on 13 family members (6 affected and 7 normal) under the assumption of an autosomal dominant gene with a frequency of 10^{-4}, equal female and male recombination rates, and penetrance of 100 and 90%.

Muscle Biopsy

A muscle sample from one affected male was obtained through biceps biopsy (after informed consent), frozen in liquid nitrogen immediately after removal, and stored at −70°C until use. Routine standard histological (HE, modified Gomory) and histochemical analysis (ATPases, NADH, and SDH) was performed. Protein analysis was done by double immunofluorescence (IF) labeling reaction on frozen sections and Western blot (WB) as described elsewhere [Ho-Kim et al., 1991; Vainzof et al., 1991]. For beta1-syntrophin analysis, rabbit anti-beta1-syntrophin (SYN35) was prepared against a peptide corresponding to amino acids 220–240 of human beta-syntrophin (M.F. Peters and S.C. Froehner, unpublished results). The other antibodies used were monoclonal anti-dystrophin C-terminal antibody Dy8/6C5 (kindly provided by Dr. L.V.B. Anderson, Newcastle, UK), N-terminal of dystrophin (rabbit 303-8, kindly provided by Dr. Jeff Chamberlain), rod-domain of dystrophin (monoclonal Dy4/6D3), monoclonal Ad1/20A6 (for α-SG), monoclonal 35DAG/21B5 (for γ-SG; Sewry et al., 1996), and α2-laminin (Gibco, Grand Island, NY) for merosin.

RESULTS

Clinical and Family Analysis

The proposita, currently 30 years old, stated onset at age 18 with gait disturbance. Other family members referred onset between 18 and 26 years (mean 21.2 ± 3.2). Two affected relatives died, respectively, at age 68 (II-2) and 65 (II-3) of cardio respiratory complications. Individual III-30 had an accidental death at age 30. Three women (II-2, III-1, and III-3) were wheelchair bound, respectively, at age 40, 33, and 40. One man (III-6, age 50) and one woman (III-9, age 44) can walk only short distances with the help of a cane. One individual in generation II (already deceased) was said to be wheelchair-bound around age 40.

Clinical assessment showed a spastic gait in all ambulatory patients. Neurological examination revealed grade 4 hyperreflexia in lower and upper limbs, bilateral Babinsky, clonus and weakness of hip flexion, and ankle dorsiflexion. All patients complained of bladder disfunction.

The proportion of affected :unaffected individuals in generations II and III did not differ from expected for autosomal dominant inheritance with complete penetrance (11/18; $P > 0.05$). No instance of incomplete penetrance was observed for the older generations (II and III in the pedigree).

Linkage Analysis

As shown in Table I, linkage to microsatellite polymorphisms flanking the 2p, 14q, and 15q loci showed

TABLE I. Two Point Lod Scores Between Microsatellites of Chromosomes 2p, 14q, 15q, and 8q in a Brazilian Pedigree With Autosomal Dominant Pure HSP

		Recombination fraction				
Penetrance	Locus	0.00	0.01	0.05	0.10	0.2
100%	D2S2255	−INF	−7.19	−3.80	−2.42	−1.18
90%		−7.37	−5.32	−3.07	−2.05	−1.04
100%	D2S2283	−INF	−4.11	−2.08	−1.27	−0.53
90%		−6.70	−2.36	−1.41	−0.91	−0.4
100%	D2S352	−INF	−1.40	−0.72	−0.44	−0.20
90%		−5.39	−1.41	−0.72	−0.45	−0.20
100%	D2S2347	−INF	−1.41	−0.72	−0.45	−0.20
90%		−5.39	−1.41	−0.72	−0.45	−0.20
100%	D2S367	−INF	−8.41	−4.32	−2.66	−1.16
90%		−6.90	−5.73	−3.31	−2.14	−0.97
100%	D14S266	−INF	−5.31	−2.60	−1.52	−0.57
90%		−6.23	−3.40	−1.84	−1.12	−0.42
100%	D14S288	−INF	−3.20	−1.23	−0.51	0.01
90%		−6.03	−2.36	−0.97	−0.41	0.01
100%	D14S269	−INF	−7.01	−3.61	−2.23	−0.99
90%		−6.46	−4.26	−2.54	−1.66	−0.79
100%	D14S978	−INF	−4.90	−2.23	−1.20	−0.37
90%		−6.05	−2.00	−1.05	−0.57	−0.12
100%	D14S989	−INF	−4.72	−2.07	−1.05	−0.23
90%		−6.90	−1.82	−0.88	−0.41	0.01
100%	D15S128	−INF	−3.02	−1.07	−0.36	0.14
90%		−4.90	−3.22	−1.27	−0.54	−0.01
100%	D15S986	−INF	−3.00	−1.67	−1.15	−0.66
90%		−6.03	−3.07	−1.73	−1.19	−0.69
100%	D15S975	−INF	−1.58	−0.89	−0.60	−0.32
90%		−5.19	−1.58	−0.89	−0.60	−0.32
100%	D15S165	−INF	−3.00	−1.67	−1.15	−0.66
90%		−6.03	−3.07	−1.73	−1.19	−0.69
100%	D8S200	−INF	−2.73	−0.79	−0.10	0.34
90%		−6.89	−1.89	−0.53	0.00	0.33
100%	D8S85	−INF	−3.14	−1.19	−0.46	0.05
90%		−7.49	−2.31	−0.92	−0.37	0.03
100%	D8S199	−INF	−1.21	0.03	0.44	0.61
90%		−6.30	−0.38	0.30	0.53	0.59
100%	D8S198	−INF	0.96	1.48	1.53	1.30
90%		−5.87	0.76	1.28	1.35	1.16
100%	D8S586	−INF	0.66	1.20	1.30	1.14
90%		−4.68	0.50	1.05	1.15	1.01
100%	D8S1804	3.31	3.26	3.04	2.76	2.15
90%		3.10	3.05	2.85	2.58	2.00
100%	D8S1774	3.01	2.96	2.75	2.49	1.91
90%		2.80	2.80	2.56	2.30	1.76
100%	D8S568	3.01	2.96	2.75	2.49	1.91
90%		2.80	2.80	2.56	2.30	1.76

negative lod scores excluding these regions as candidate genes. Eight markers flanking the candidate region for the SPG8 locus were used. Positive lod-scores were obtained with markers to the SPG8 locus (maximum two point Lod score Zmax = 3.1 at theta = 0 for D8S1804; Zmax = 2.8 at theta = 0 for D8S1774 and D8S568), suggesting linkage to this locus.

The genotype for all markers flanking the candidate region for the SPG8 locus are illustrated in Figure 1. Three obligate recombinants were identified (individuals III-15 and IV-5 who are affected and III-12 who is non-affected). These results are consistent with those reported by Hedera et al., [1999a] but did not permit the narrowing down of the candidate region, which was subsequently reduced from 6.2 cM to 3.4 cM in the kindred described by Reid et al. [1999a].

Beta 1 Syntrophin Analysis

β1-syntrophin immunofluorescence analysis showed a positive sarcolemmal labeling pattern in the patients and in normal controls, but with a weaker intensity when compared to the dystrophin reaction. Western blot analysis showed a band with a comparable intensity and with the same apparent molecular weight (approximately 59 kDa) in the patient and in three additional control muscles (Fig. 2B). In addition, a strong positive sarcolemmal labeling pattern was observed for the antibody for the N- and C-terminal domains of dystrophin, as well as for α-SG, γ-SG, and merosin (Fig. 2C).

Morphological and histochemical muscle analysis demonstrated a normal pattern with no presence of ragged red fibers nor SDH altered stained fibers, which could reflect mitochondrial abnormalities. In some regions, mild type grouping was observed (Fig. 2).

DISCUSSION

Among the pure forms of inherited spastic paraplegia, the SPG4, linked to chromosome 2p, is the most prevalent accounting for 40–50% of the reported cases [Fink et al., 1996; Hazan et al., 1999a]. The SPG3 (at 14q) and SPG6 (at 15q) are rarer and represent less than 10% of the pure AD forms [Fink et al., 1996].

Two families (a North American family of German descent and a Scottish kindred) were found to be linked to a locus on chromosome arm 8q23–24 [Hedera et al., 1999; Reid et al., 1999a] and classified as SPG8. However, the relative frequency of this form of AD spastic paraplegia is not known and the existence of at least a sixth locus is demonstrated by several families for whom the known loci have been excluded [Reid et al., 1999b]. We are reporting for the first time pure AD inherited spastic paraplegia linked to the SPG8 locus in a non-consanguineous family of African-Brazilian descent.

A great intrafamilial and inter familial clinical variability has been reported for autosomal dominant spastic paraplegia. For SPG4, both complicated and pure

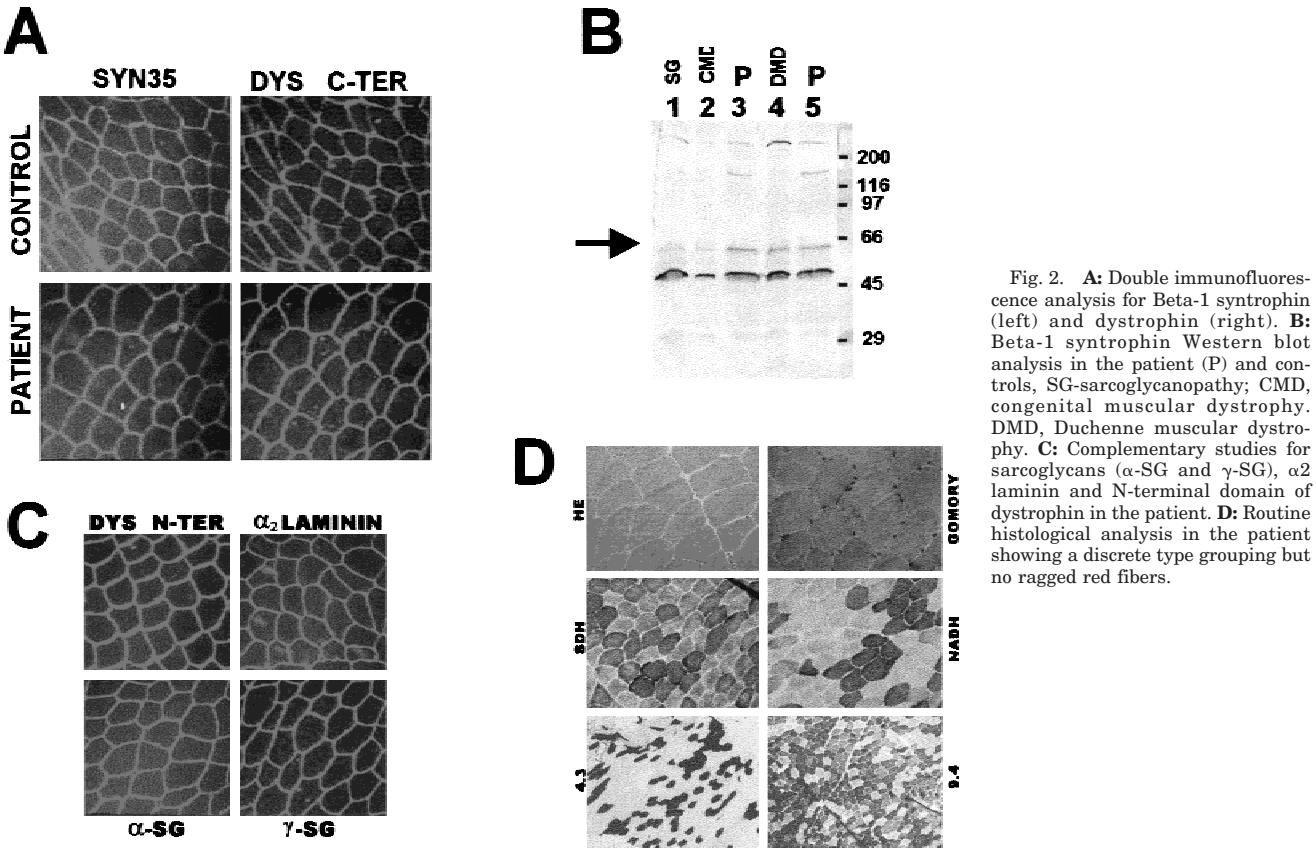

Fig. 2. **A:** Double immunofluorescence analysis for Beta-1 syntrophin (left) and dystrophin (right). **B:** Beta-1 syntrophin Western blot analysis in the patient (P) and controls, SG-sarcoglycanopathy; CMD, congenital muscular dystrophy. DMD, Duchenne muscular dystrophy. **C:** Complementary studies for sarcoglycans (α-SG and γ-SG), α2 laminin and N-terminal domain of dystrophin in the patient. **D:** Routine histological analysis in the patient showing a discrete type grouping but no ragged red fibers.

forms have been mapped to the same locus (Henzlef et al., 1998).

The clinical course of the two SPG8 families of European descent is severe. Indeed, 10 of 15 subjects in Hedera's kindred and 6 of 15 in the family reported by Reid et al. [1999a] were wheelchair-bound. Interestingly, although in the Brazilian family the mean age at onset occurred earlier (21.2 ± 3.2 in our family, 37.3 ± 12.2 in Hedera's kindred, and 29.6 ± 11.0 in Reid's family), wheelchair confinement or inability to walk independently was comparable (four among seven subjects older than 40 in our family), suggesting a slower progression after onset. On the other hand, one individual was reported as asymptomatic in each of the two European families, while no instance of incomplete penetrance was observed in the present family. Comparison with other pure forms of SPG showed that phenotype in the present family is more severe, considering both age at onset and clinical progression than the one observed in two large Brazilian SPG4 and X-linked kindreds [Zatz et al, 1995; Rocco et al., 1998].

Clinical anticipation has been reported for 14q [Gispert et al., 1995] and SPG4 kindreds by Nielsen et al. [1997], who reported CAG repeat expansion in six Danish families. However, clinical anticipation is apparently an infrequent finding [Fink et al., 1996] and recent studies excluded expanded CAG repeat in a new series of SPG4 families [Hazan et al., 1999b]. In the present family comparison between subject II-2 and her offspring as well as individuals III-3 and IV-2 does not suggest clinical anticipation in accordance with Hedera's family.

Recently, mutations in a novel gene, paraplegin, were found in patients affected by autosomal recessive HSP linked to 16q24 [Casari et al., 1998]. Paraplegin has high homology to yeast mitochondrial adenosine triphosphatases, with both proteolytic and chaperon-like activities at the inner mitochondrial membrane. Quadriceps muscle biopsy of HSP subjects with paraplegin gene mutations revealed ragged red fibers and cytochrome c oxidase (COX)-negative indicative of mitochondrial abnormality. However, it is not known whether mitochondrial disturbance is uniquely associated with mutations in the paraplegin gene or if it also occurs in other forms of HSP. In accordance with the results observed by Hedera et al. [1999b], the muscle biopsy in one affected male from the present family showed no mitochondrial abnormalities, thus reinforcing that mitochondrial abnormalities are not primary features of SPG8.

Several genes were mapped to the SPG8 region on chromosome 8q23–24. Among them, beta-1 syntrophin, a dystrophin associated protein [Ahn et al., 1996; Peters et al., 1997] represented a good functional and positional candidate. As with all of the syntrophins, beta-syntrophin is thought to link signaling proteins (ion channels, kinases, nitric oxide producing enzymes, etc.) to the dystrophin/utrophin complexes and thus target these signaling systems to membrane specializations. This probable function, along with the widespread expression of beta1-syntrophin in many tissues including the nervous system and muscle, made it a functional candidate for the disease under investigation. However, syntrophin analysis in muscle by immunofluorescence and Western blotting from patient IV-2 showed an apparently normal pattern.

An apparently normal expression of the primary product of a gene carrying a pathogenic mutation has been reported previously for other genes, such as dystrophin in Becker muscular dystrophy. Therefore, the apparently normal syntrophin beta1 expression cannot definitively exclude this gene as the cause of SPG8. However, it is unlikely that the deficiency of this protein is responsible for the abnormal phenotype in the present family.

In conclusion, we are confirming linkage to the SPG8 locus in an African-Brazilian family with pure autosomal dominant spastic paraplegia. Our results also suggest that beta-1 syntrophin is unlikely to be a candidate gene for SPG8. The identification of the disease-causing gene will be very important to elucidate the pathological mechanisms responsible for this form of spastic paraplegia.

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